



Letter to the Editor

A ferromagnetic model for the action of electric and magnetic fields in cryopreservation[☆]Atsuko Kobayashi^{a,*}, Joseph L. Kirschvink^b^a Earth-Life Science Institute, Tokyo Institute of Technology, 2-12-1 IE-31, Ookayama, Meguro, Tokyo 152-8551, Japan^b Division of Geological and Planetary Sciences, California Institute of Technology 170-25, Pasadena, CA 91125, USA

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ABSTRACT

Recent discussions in the literature have questioned the ability of electromagnetic exposure to inhibit ice crystal formation in supercooled water. Here we note that strong electric fields are able to disrupt the surface boundary layer of inert air on the surface of materials, promoting higher rates of heat transport. We also note that most biological tissues contain ferromagnetic materials, both biologically precipitated magnetite (Fe₃O₄) as well as environmental contaminants that get accidentally incorporated into living systems. Although present at trace levels, the number density of these particulates is high, and they have extraordinarily strong interactions with weak, low-frequency magnetic fields of the sort involved in claims of electromagnetic cryopreservation. Magnetically-induced mechanical oscillation of these particles provides a plausible mechanism for the disruption of ice-crystal nucleation in supercooled water.

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Ice expands ~10% upon freezing when it crystallizes, which can destroy cell membranes by the simple expansion effect, coupled with the damaging effect of sharp edges from growing ice crystallites. This can be avoided by supercooling the water, chilling it to a glassy state that does not go through the expansion process, and/or limiting the size of the ice crystals that do form. During the last 10 years the ABI Corporation (Chiba, Japan) has marketed a “Cells Alive System”, CAS, which claims to have improved the ability of much larger volumes of animal and vegetable matter to be frozen with minimal damage to cellular ultrastructure from ice crystal growth. The programmable CAS freezers expose samples to low-frequency oscillating electric and magnetic fields while controlling the supercooling of the materials in the critical 0 °C to −20 °C temperature interval by blowing refrigerated air on the samples [18,34,35]. Published analyses suggest this technology can aid in the freezing and shipping of delicate fruits and vegetables, in the enhanced cryopreservation of human transplant tissues like teeth [1,28] and embryonic stem cells [29], and even promotes whole-organism survival of frozen small animals like *Drosophila* [33].

Biophysical theory

Papers and patents published by the ABI group [17,18,34,35] postulate mechanisms of action that do not agree with basic biophysics. In particular, the oscillating electric and magnetic fields are supposed to ‘wiggle’ water molecules directly to inhibit the nucleation of ice crystals in the supercooled state, as well as to promote rapid and isothermal cooling of the sample interiors.

Wowk [44] pointed out in a recent critique that water molecules are diamagnetic, and will not produce any effect above thermal noise when exposed to the weak oscillating magnetic fields (<10 Gauss or 1 mT) used in the CAS freezers. He also notes that electric fields are known to either inhibit or enhance ice crystal formation slightly, depending upon the conditions used, but not at the levels claimed for these devices. In a direct test of the magnetic aspect of the CAS freezers, Suzuki et al. [38] also report that the oscillating magnetic field treatments *alone* did not alter the cooling time curves for test samples of radish or sweet potatoes, and had no visible effect on cellular microstructures of the tissues they examined.

We would like to point out that two themes of our previous research (*electrostatic effects on heat transport*, and *biologically-precipitated ferromagnetic materials*) happen to have a direct bearing on possible solutions to the biophysical puzzle as to why this freezing technology might work.

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Electrostatic effects on heat transport

In the first of these [26], we studied the effect of electrostatic fields on the rate of drying of wet materials. It is well known from the study of transport phenomena that a thin layer of relatively inert air exists at the surface of most materials where the relative velocity of gas flow asymptotically drops to zero. These surface boundary layers both interfere with the diffusion of gases out of the material and limit the rate of convective heat transfer into it (e.g., [3,6,7]). It is also known that an electric or “corona” wind is generated on the surface of electrically charged objects as a result of ions leaving the surface, and this wind can cause a marked increase in heat conduction at a surface by disrupting the stagnant surface boundary layer [2,4,8,27,31,37]. This electrostatic effect per ion is several orders of magnitude above thermal noise. In our previous study, we found that electrostatic fields comparable to those used in CAS freezers were able to disrupt the inert surface boundary layer of air molecules, and dramatically shorten drying times [26]. We therefore argue here that the high-voltage electrostatic fields applied in the CAS freezers are increasing the cooling efficiency by disrupting the surface boundary layer of inert gas at the surface of their materials. The cooling enhancements shown by Owada et al. [34] are, in fact, similar in style to that we reported previously [26]. Hence, either DC or AC high-voltage electric fields would be expected to promote rapid heat removal needed for supercooling.

Biologically-precipitated ferromagnetic materials

An intrinsically more interesting question concerns the possible mechanism of action of the weak, oscillating magnetic fields on cryopreservation. There are only four possible physical coupling mechanisms that can yield interaction effects of oscillating magnetic fields with matter (electrical induction, diamagnetism, paramagnetism, and ferromagnetism). However, for low-frequency fields weaker than a few hundred uT, all except ferromagnetism do not work, with peak interaction energies well below the thermal noise limit. We are in complete agreement with Wowk [44] on this. However, particles of *ferromagnetic* materials can interact hundreds to thousands of times stronger with earth-strength magnetic fields than the background thermal energy (see discussion by Kirschvink [19]). Owada et al. [34,35] and Wowk [44] did not consider the well-known presence of ferromagnetic materials, principally biologically-precipitated magnetite (Fe_3O_4), in a wide range of biological tissues (see [13,20,30,39–41,43], for example). These observations have been replicated widely (e.g., [5,9,11,14–16,36]). Brain tissues in humans have been studied extensively [5,9–11,16,21,22,36], and magnetite deposits in specialized cells are extensive [24,25]. On occasion, other ferromagnetic materials of inorganic origin, which are also ubiquitously present in the environment [23], can even work their way into biological tissues [42]. Typical animal tissues have background concentrations of ferromagnetic materials in the 1–1000 ng/g range, with average levels of ~4 ng/g.

A recent high-resolution study of magnetoreceptor cells containing biological magnetite in fish by Eder et al. [12] demonstrated that the individual cells are surprisingly magnetic (up to 100 fA m^2), with magnetite concentrations often 100 times greater than typical cells of magnetotactic bacteria. These cells have interaction energies of up to 1500 times larger than the background thermal noise (kT, where k is the Boltzmann constant and T the absolute temperature) in the geomagnetic field, which would be on the order of 4500 times larger than kT in the typical magnetic fields (0.15 mT) used in the CAS freezers [18]. In our work on human tissues [21], we reported the presence of ~4 ng/g of magnetite

in the cortex and cerebellum (with a factor of 10 \times larger in the meninges), values similar to that measured with superconducting magnetometry in a variety of other animal tissues [20]. With these measured Vertebrate cell concentrations, this yields minimum estimates of nearly 100,000 of these magnetic clusters per gram of typical tissue. In turn, this implies that the average distance of any cell within a magnetite-bearing tissue would on the order of 20 μm from a ferromagnetic cluster. Smaller particle sizes would imply correspondingly more particles, and shorter distances, from the nearest cluster.

Discussion

It seems most likely that the *electrostatic* enhancement observed during the CAS freezing process is a simple disruption of the surface boundary effect of inert air, and a more efficient heat transport process. The enhanced removal of heat from the tissues may be one factor in producing the supercritical cooling observed. In their attempt to test components of the CAS hypotheses, Suzuki et al. [38] were able to refute claims that the *magnetic* treatment was involved with heat transport. We concur with their analysis, but suggest that the *electric* exposure, not the *magnetic* exposure, is responsible for that aspect.

If the oscillation of sub-micron ferromagnetic particles distributed through tissues is involved in the reported action of CAS freezers, then we see two possible mechanisms for this inhibition of ice crystal nucleation. First, and most obvious, is the possibility that these particles normally act as some of the nucleation sites for the formation of ice crystals. Oscillations would then tend to inhibit the aggregation of the few hundred water molecules involved in the early crystal growth (e.g., [32]). This could certainly be tested experimentally. Second, the low-frequency acoustic waves from the oscillating particles will radiate outwards from the magnetite-containing cells. Unlike acoustic waves from outside the object (which can trigger ice crystal nucleation), close to the oscillating ferromagnetic particles the acoustic waves should dissipate rapidly into the surrounding tissue, with spatially large gradients. These gradients may act to disrupt the aggregates of water molecules that organize into ice crystal nucleation structures [32] by differentially shearing them apart. In either of these situations, the mechanical coupling of the ferromagnetic clusters to the surrounding cytoplasm would be an important feature for transducing the magnetic energy to the adjacent tissue.

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